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16. ABSTRACT

Sensitivity, expressed as the 96-h LC50 derived from acute lethality tests, was compared for four ages (day-of-hatch, 7-d, 14-d, and 28-d) of three atherinid fishes: Leuresthes tenuis (California grunion), Menidia menidia (Atlantic silverside), and Menidia peninsulae (tidewater silverside). Responses of each age-species combination exposed to the organophosphate insecticide, chlorpyrifos, and the carbamate herbicide, thiobencarb, were compared in both static and flowing seawater toxicity tests. Chlorpyrifos was highly toxic to all atherinids (96-h LC50's ranged from 0.4 to 6.7 µg/L); toxicity of thiobencarb was approximately two orders of magnitude lower (LC50 values from 199 to 1405 µg/L). Acute lethal responses to each pesticide were similar among the three species. Sensitivity was generally highest for 7-d and 14-d age groups, and flowing-water tests were more sensitive measures of toxicity than static tests, especially for chlorpyrifos.

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Comparative Acute Sensitivities of Early Life Stages of Atherinid Fishes to Chlorpyrifos and Thiobencarb[1]

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Abstract. Sensitivity, expressed as the 96-hr LC50 derived from acute lethality tests, was compared for four ages (day-of-hatch, 7-day, 14-day, and 28-day) of three atherinid fishes: *Leuresthes tenuis* (California grunion), *Menidia menidia* (Atlantic silverside), and *Menidia peninsulae* (tidewater silverside). Responses of each age-species combination exposed to the organophosphate insecticide chlorpyrifos and the carbamate herbicide thiobencarb were compared in both static and flowing seawater toxicity tests. Chlorpyrifos was highly toxic to all atherinids (96-hr LC50's ranged from 0.4 to 6.7 μg/L); toxicity of thiobencarb was approximately two orders of magnitude lower (LC50 values from 199 to 1,405 μg/L). Responses to each pesticide were similar among the three species. Sensitivity was generally highest for 7-day and 14-day age groups, and flowing water tests were more sensitive measures of toxicity than static tests, especially for chlorpyrifos. Comparisons of three computational methods indicate that probit and moving average methods calculate comparable LC50 estimates with the binomial method being the least uniform point estimator.

Three atherinid fishes, the California grunion (*Leuresthes tenuis*), Atlantic silverside (*Menidia menidia*), and tidewater silverside (*Menidia peninsulae*) are abundant in coastal waters and estuaries of the United States. The California grunion is an important recreational fish occurring from Monterey Bay, California, to Bahia Magdalena, Baja California Sur (Moffatt and Thomson 1975). It spawns in sand substrate at the approximate time of new and full moons from February through August (Clark 1925; Walker 1952). After about two weeks, developed embryos hatch when washed out of the sand by the next series of high tides (Middaugh *et al.* 1983; Shepard and LaFond 1940).

The Atlantic silverside (*Menidia menidia*) is a common resident of East Coast estuaries (deSylva *et al.* 1962; Dahlberg 1972; Cain and Dean 1976). It ranges from the Magdalen Islands, Canada, to northern Florida (Cox 1921; Gosline 1948; Johnson 1975). Maximum intensity spawning runs of the Atlantic silverside, like the California grunion, occur every two weeks at the approximate time of new and full moons (Middaugh 1981).

The tidewater silverside (*Menidia peninsulae*) is an estuarine species with disjunct distribution from Daytona Beach, Florida, to Horn Island, Mississippi and from Galveston Bay, Texas, to Tamiahua, northern Veracruz, Mexico (Johnson 1975; Chernoff *et al.* 1981). It is typically found at intermediate to high salinities (15 to 30‰). While little is known of the reproductive biology of the tidewater silverside, preliminary field observations suggest that, like the California grunion and Atlantic silverside, it is also a cyclic spawner.

Current literature indicates that standardization of acute testing procedures for aquatic species requires identification of sources of variability that affect test organism sensitivity (Roberts *et al.* 1982). For example, acute toxic responses among three age groups of laboratory-reared fathead minnow (*Pimephales promelas*) fry exposed to nine organic chemicals differed by factors of 1.1 to 4 (average = 1.7) (Mayes *et al.* 1983). While no difference in sensitivity was observed among sheepshead minnow

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(*Cyprinodon variegatus*) fry, juveniles, and adults exposed to endrin (Hansen *et al.* 1977), 4 age groups of laboratory-reared sheepshead minnows exposed to pentachlorophenol in acute tests demonstrated a 1.8 fold difference in sensitivity (Borthwick and Schimmel 1978).

To determine the toxicity of two 3rd generation pesticides to 4 age groups of 3 ecologically important atherinid fishes, we tested chlorpyrifos a widely used multi-purpose organophosphate insecticide, and thiobencarb a relatively new carbamate herbicide used to control weeds in rice. The half-lives of chlorpyrifos and thiobencarb in untreated sediment-water studies were 24 and 6.4 days, respectively. Chlorpyrifos may pose a hazard to sediment-dwellers due to its persistence and high acute toxicity. However, thiobencarb is less toxic, less persistent and less hazardous to aquatic organisms (Schimmel *et al.* 1983). Current registration labels state the precaution "This product is toxic to fish . . .", however we are aware of no documented fish kills or aquatic environmental problems caused by use of products containing either chemical. Both were selected for testing based on usage.

Chlorpyrifos [*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridinyl) phosphorothioate] is the active ingredient in several formulations (granules, dusts, and emulsifiable concentrates) designed to control an array of insect pests including fire ants, turf and ornamental plant insects, mosquitoes, cockroaches, termites, lice, and hornflies. In the United States, foliar application to cotton, peanuts, and sorghum constitutes the major agricultural usage. Chlorpyrifos protects southern pine seedlings from pales and pitch eating weevils and pine trees from damage by southern pine beetles. In other countries, applications include use of the emulsifiable concentrate for control of insect pests in households and on domestic animals, as well as insect pests in lowland rice, vegetables and field crops (Farm Chemicals 1984).

Thiobencarb [*S*-(4-chlorophenyl)methyl] diethylcarbamothioate is applied directly to water-seeded rice (California only), dry-seeded rice (Texas, Louisiana, Mississippi, and Arkansas) and transplanted upland rice as a broad spectrum preemergence and early postemergence herbicide for control of grasses and broadleaf weeds (Farm Chemicals 1984). More than 40 countries used approximately one million kg of technical material in 1980, and producers are awaiting full registration by the US Environmental Protection Agency (EPA) for use of thiobencarb on domestic rice (Anonymous 1981). In the United States, rice is usually cultivated near coastal waters so that paddies can be irrigated by controlled tidal flooding. Schimmel *et al* (1983) calculated that water-seeded rice fields flooded with 6 inches of water and treated following label directions (4.0 lb/A) would result in thiobencarb concentrations (~3 mg/L) hazardous to aquatic animals present. Suzuki *et al.* (1977) detected thiobencarb concentrations between 10 and 0.1 µg/L in rivers and agricultural drainages following overflows from rice paddies. Exposure of atherinid fishes in rice fields may be of interest since the inland silverside (*Menidia beryllina*) was considered for introduction as a larvivorous mosquito predator in California rice fields (Cech and Moyle 1983). Middaugh *et al.* (In review) found *Menidia beryllina* to be an efficacious predator for control of pestiferous and disease vector mosquitos in brackish water impoundments in Florida and in Arkansas rice fields. Figure 1 shows geographic use patterns for chlorpyrifos and thiobencarb in coastal states and delineates the biogeographic distribution of the fish species used in this study.

Four age-groups (day-of-hatch, 7-day, 14-day, and 28-day posthatch) of each of the three species were tested to determine differences in sensitivity to chlorpyrifos and thiobencarb due to age during the early life histories of the fishes. Responses of the ages and species in static and flowing-water 96-hr acute lethality tests were analyzed statistically.

Finally, we compared the three computational method's (probit, moving average, and binomial) point estimates (96-hr LC50's) calculated using these empirical concentration-mortality data sets.

Materials and Methods

California grunion (*Leuresthes tenuis*) eggs were stripped from adults during nighttime spawning runs at Black's Beach, La Jolla, CA (32°52'37" lat., 117°15'0" long.), from March through June of 1981 and 1982. Eggs from 10 to 15 females were stripped into a glass culture dish of seawater and fertilized with sperm from 5 to 7 males. After approximately 15 min, fertilized eggs were washed several times with seawater from the collection site and 2 to 3 thousand pipetted into each of four 500 ml glass vacuum bottles. Eggs were shipped, air express, from California to the Environmental Research Laboratory at Gulf Breeze, Florida. Upon arrival, four clusters of 200–300 eggs were pipetted into shallow depressions (2–4 mm deep) in clean, dry beach sand in 20 cm diameter glass culture dishes. Each group of eggs was covered with a layer of dry sand. Then, 20 to 30 ml of seawater was pipetted onto the sand where eggs were buried. The culture dishes, each covered with a glass plate to inhibit evaporation, were placed in a dark incubator at 25°C. After 13 to 14 days, developed eggs were removed from the sand and placed in a culture dish containing seawater at 25°C and 30‰. Mild agitation of the water promoted hatching of larvae. Grunion larvae were then poured gently from the hatching dish into a 160 L all-glass aquarium that received 20 L of filtered seawater/hr at 25 ± 1°C and 25 to 28‰. Densities of grunion ranged from 3

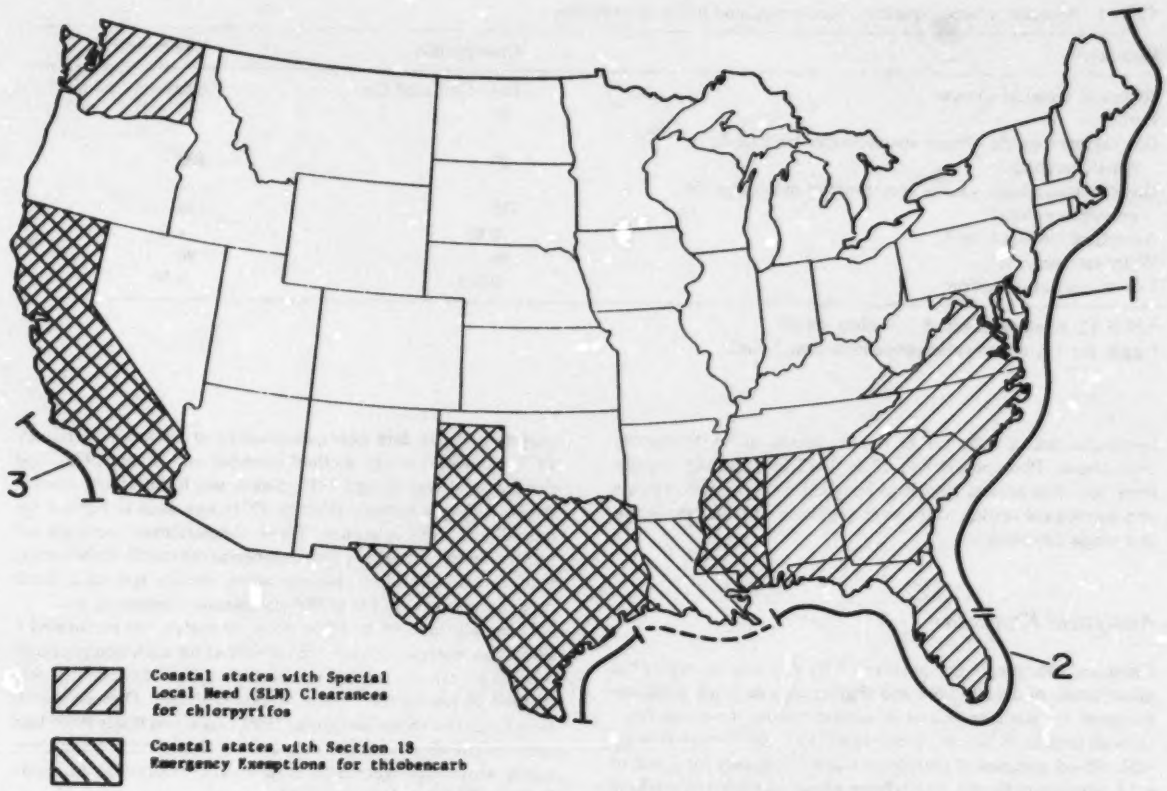
1
2
3
Coastal states with Special Local Need (SLN) Clearances for chlorpyrifos
Coastal states with Section 18 Emergency Exemptions for thiobencarb

Fig. 1. Biogeographic distribution of atherinid fishes used in comparative toxicology studies and coastal states using chlorpyrifos and thiobencarb pesticides. 1 Atlantic Silverside, Maine to Florida. 2 Tidewater Silverside, Daytona Beach, Florida to Horn Island, Mississippi . . . and Galveston Bay to Mexico. 3 California Grunion, San Diego to Monterey, California

to 6/L of seawater. They were maintained under a 14 hr L: 10 hr D photoperiod (illumination = 11,000 lux) and fed *Artemia* nauplii, 5,000 to 10,000/L, twice daily (0700–0900 and 1500–1600) until used in toxicity tests.

Eggs of Atlantic silverside, *Menidia menidia*, and tidewater silverside, *Menidia peninsulae*, were obtained from adults induced to spawn in the laboratory. Forty to 60 sexually mature individuals of each species (sex ratio 1:1) were maintained in separate spawning systems. Each recirculating system consisted of two 1.3 m diameter spawning tanks with water maintained at a depth of 45 cm, a temperature of 25 ± 1°C and 30‰ salinity.

Spawning in each species was induced by controlled photoperiod and simulated tidal periodicity. A detailed description of the spawning system and responses of each species to environmental cues is given by Middaugh *et al.* (1985) and Middaugh and Hemmer (1984).

Fertilized Atlantic and tidewater silverside eggs, with small portions of the polyester spawning substrate, were maintained in aerated 10 L wide-mouth glass separatory funnels containing 7 L of seawater at 25 ± 1°C and 20‰ salinity. Hatching occurred 6 to 7 days after fertilization. Larvae, stocked at a density of 10 to 20/L, were fed the marine rotifer *Brachionus plicatilus* at a density of 5,000 to 10,000/L twice daily (0700 to 0900 and 1500 to 1600) for 4 to 6 days. Three days (Atlantic silverside) or 4 days (tidewater silverside) after hatching, young (<8 hr old) *Artemia* nauplii were provided in combination with the rotifers. The density of both species of *Menidia* was reduced to 5 to 10/L at 7 days post hatch. Thereafter, they were fed only *Artemia* nauplii at 5,000 to 10,000/L twice daily.

Acute Toxicity Texts

Static- and flowing-water 96-hr acute lethality tests consisted of both solvent and seawater control treatments and a geometric series of five exposure concentrations. Ten randomly assigned fish per treatment for static and twenty per treatment for flow-through tests provided quantal concentration-mortality data sets for analysis. Test protocols as described by ASTM (1980) were employed with the exception that marine rotifers or *Artemia* nauplii or both were fed to grunion and silversides during the 96-hr exposure periods. We fed 10 to 20 live food organisms per fish 2 to 3 times a day during each exposure to provide minimal nutrition without confounding results due to dissolved oxygen or loading problems.

All tests were conducted at 25°C, using a constant temperature bath. Salinity was controlled at 20‰ for *M. menidia* and *M.*

Table 1. Pesticide sources, purities, recoveries, and limits of detection

Parameter	Chlorpyrifos	Thiobencarb
Technical material source	Dow Chemical Co	Chevron Chemical Co
Purity, %	92	90
Gas chromatograph—mass spectrometer base peak, mass/charge ratio	97	100
Gas chromatograph—mass spectrometer molecular ion, mass/charge ratio	351	258
Analytical standard, ng/L	0.03	1.0
Water recovery, %[a]	96	96
Lower limit of detection[b]	0.015	0.50

[a] N > 12; relative standard deviation <9.6%
[b] µg/L for 1 L of seawater concentrated to 2.0 mL

peninsulae and at 25‰ for *L. tenuis*, due to niche differences cited above. Photoperiod was 12 hr L:12 hr D during toxicity tests. All other factors were held constant within practical limits of toxicological testing to increase precision of tests conducted at a single laboratory.

Analytical Procedure

Chemical analyses were performed by gas chromatographic quantitation of chlorpyrifos and thiobencarb in stock solutions prepared for static tests and in seawater from the acute flow-through tests at 48 and 96 hr. Seawater (1 L) was extracted twice with 100-ml portions of petroleum ether by shaking for 1 min in a 2-L separatory funnel. The solvent phase was dried by passage through a funnel containing heat-treated (600°C) glass wool and collected in a 250-ml Kuderna-Danish concentrator. The concentrator was fitted with a Snyder column and the solvent evaporated on a steam cabinet to about 20 ml, followed by concentration with a nitrogen evaporator at room temperature to not less than 2 ml. The lower limits of detection and recovery data for fortified samples are given in Table 1.

Analytical Equipment: Chlorpyrifos and thiobencarb analyses were performed with a Hewlett-Packard Model 5730A gas chromatograph equipped with a dual nitrogen-phosphorus flame ionization detector. Detector gases were hydrogen at 4 ml/min and air at 100 ml/min. Operating conditions were: flow rate of helium carrier gas, 30 ml/min; injector and column temperature 200°C; and detector temperature, 300°C. The column was 182 cm by 2 mm i.d. glass, packed with 5% QF-1 on 80-100-mesh Gas-Chrom Q.

All chemicals were quantitated by peak area, using the Hewlett Packard Model 3353E Laboratory Data System. Aliquots of 5 µl were injected and compared to the analytical standard concentration noted in Table 1.

Statistical Methods

Individual concentration-mortality data sets from static (nominal concentrations) and flowing-water (measured concentrations) 96-hr acute lethality tests were subjected to three methods of analysis to derive the 96-hr LC50 and 95% confidence interval. Median lethal concentrations (Table 2) for each set of concentration-mortality data were calculated by probit analysis (Finney 1971), moving average method (Kendall and Stuart 1973), and the binomial test (Siegel 1956; Sokal and Rohlf 1969), respectively. Abbott's formula (Finney 1971) was used to correct for mortality in control groups. These computational methods are used routinely at EPA's Environmental Research Laboratory, Gulf Breeze, Florida to analyze acute toxicity test data. Each procedure meets ASTM (1980) acceptability requirements.

Using all three 96-hr LC50 point estimates, we performed a three-way analysis of variance (ANOVA) for each compound to determine effects related to species, age of test animal, and method of testing (*i.e.*, static or flow-through). Duncan's multiple range test (Steel and Torrie 1960; Sokal and Rohlf 1969) was used for pairwise *post hoc* comparisons. Both ANOVA and Duncan's procedure were executed by the Statistical Analysis System (SAS Institute Inc. 1982).

Our basic ANOVA hypothesis can be expressed as a simple model equation which describes the basic components of the 96-hr LC50 or response variable (y):

$$y = \text{mean effect } (\mu) + \text{species (S) effect} + \text{age (A) effect} + \text{method (M) effect} + SA + SM + AM + \text{error term } (\epsilon)$$

where SA, SM, and AM represent two-way interactions of effects which must be tested in this experimental design.

Results

Table 2 includes the 95% confidence limits for each 96-hr LC50 to describe the distribution of mortality data. However, only the point estimate (the 96-hr LC50) was compared in the ANOVA design used here. Associated confidence intervals are tabulated to provide measures of variability.

Mean toxicity (probit 96-hr LC50) of chlorpyrifos was 180 times higher than thiobencarb in static tests and 380 times higher in flow-through tests. For chlorpyrifos, flow-through test sensitivity was three times greater than static tests, and for thiobencarb, flow-through sensitivity was 1.4 times greater. Ranking *L. tenuis*, *M. menidia*, and *M. peninsulae*, respectively: mean 96-hr LC50's

Table 2. 96-hr LC50s calculated by probit, moving average, and binomial test for three atherinid fishes exposed to thiobencarb in static (S) and flow-through (FT) tests. Values are expressed in μg/L of seawater. In tests with control mortality, the data were corrected for control mortality, using Abbott's correction

Species	Age (days)	Method[a]	Chlorpyrifos			Thiobencarb		
			Probit	Moving average	Binomial	Probit	Moving average	Binomial
California grunion, *Leuresthes tenuis*	0	S	5.5(1.5–11.5)	6.0(2.7–11.1)	6.7(0–18.3)	309(248–380)	316(264–372)	269(170–700)
		FT	1.0(0.8–1.3)	1.0(0.8–1.3)	1.2(0.5–2.2)	265(250–283)	275(235–320)	267(235–310)
	7	S	2.7(2.0–3.5)	2.7(1.9–3.6)	2.8(1.2–5.5)	483(378–617)	478(379–621)	549(153–708)
		FT	1.0(0.8–1.3)	1.0(0.8–1.3)	1.0(0–1.9)	237(204–275)	235(192–281)	247(125–350)
	14	S	1.8(1.2–2.5)	1.8(0.9–2.8)	1.7(0–6.0)	586(448–738)	600(461–742)	660(0–1100)
		FT	1.0(0.8–1.4)	1.1(0.8–1.8)	0.9(0.5–2.5)	377(324–456)	394(345–451)	386(220–755)
	28	S	2.6(2.0–3.7)	2.6(2.0–3.7)	2.4(1.00–∞)	*502(408–618)	497(379–626)	522(352–977)
		FT	1.3(1.0–1.7)	1.3(1.0–1.7)	1.6(0.7–∞)	333(271–405)	327(266–402)	362(195–535)
Atlantic silverside, *Menidia menidia*	0	S	4.5(3.6–5.7)	4.4(3.7–5.4)	5.6(1.7–10.0)	458(341–640)	460(349–669)	531(216–1000)
		FT	0.5(0.4–0.7)	0.5(0.4–0.6)	0.6(0.2–1.1)	386(304–457)	332(265–419)	380(102–547)
	7	S	2.8(2.3–3.5)	2.8(2.3–3.5)	2.8(2.0–5.5)	450(347–579)	457(348–575)	400(194–1500)
		FT	1.0(0.9–1.2)	1.0(0.9–1.2)	1.0(0.7–1.4)	204(177–242)	221(190–253)	199(130–325)
	14	S	2.4(1.9–2.9)	2.3(1.8–2.9)	2.6(2.1–3.5)	633(502–813)	619(526–740)	840(288–1200)
		FT	1.1(0–∞)	1.1(1.0–1.3)	1.1(0.9–∞)	414(350–493)	397(335–494)	455(223–514)
	28	S	4.1(3.3–5.2)	4.0(3.2–5.1)	4.5(2.4–6.7)	748(542–876)	703(0–∞)	703(0–1230)
		FT	3.0(2.6–4.0)	3.1(2.7–3.7)	3.1(2.0–4.2)	675(603–781)	674(601–790)	674(547–∞)
Tidewater silverside, *Menidia peninsulae*	0	S	4.2(3.3–5.5)	4.2(3.2–5.4)	3.9(1.9–9.0)	526(442–645)	520(442–655)	560(257–750)
		FT	1.0(0–∞)	1.0(0.8–1.3)	1.1(0.3–2.5)	299(230–370)	256(191–327)	328(102–547)
	7	S	2.0(1.5–2.8)	2.0(1.5–2.7)	1.8(0.8–∞)	396(270–540)	390(236–592)	324(194–1500)
		FT	0.5(0.5–0.6)	0.5(0.5–0.6)	0.5(0.4–0.7)	464(398–574)	447(387–520)	383(344–850)
	14	S	1.8(1.5–2.2)	1.8(1.4–2.3)	1.8(1.3–3.5)	511(414–632)	511(417–628)	511(238–1100)
		FT	0.4(0.3–0.6)	0.4(0.3–0.5)	0.4(0.2–0.9)	394(304–585)	388(298–595)	455(223–514)
	28	S	3.9(3.1–5.8)	4.0(3.4–5.1)	4.0(3.0–∞)	1174(900–1621)	1159(916–1576)	1405(450–2080)
		FT	0.9(0.7–1.1)	0.9(0.7–1.1)	1.0(0.7–1.6)	817(701–1056)	854(0–∞)	865(415–∞)

* Actual control mortality (10%) was adjusted to 5% control mortality for Abbott's correction since neither probit nor moving average would calculate an LC50 for actual data

[a] S = static; FT = flow-through

(probit) for chlorpyrifos were 3.1, 3.4, and 3.0 μg/L in static tests, and 1.1, 1.4, and 0.7 μg/L in flow-through tests; for thiobencarb, this species comparison was 470, 572, and 652 μg/L in static tests, and 303, 420, and 493 μg/L in flow-through tests.

For thiobencarb, ANOVA revealed no significant ($\alpha = 0.05$) interactions among the three factors tested (Table 3). However, there were significant effects related to the main factors: age, test method, and species. Duncan's multiple range tests revealed significant pairwise differences between age groups and between species (Table 4). Duncan's test detected no statistical differences among the day-of-hatch, 7-day, and 14-day-old groups for 96-hr LC50 values calculated by probit and moving average methods, although the 28-day group was significantly less sensitive to thiobencarb than the other three age groups. The binomial 96-hr LC50 values did not differ between the 28 day group and the 14 day group (Table 4).

ANOVA for the chlorpyrifos LC50 data (Table 3) showed a significant interaction between age and method; therefore, additional separate analyses of variance were performed for static and flow-through tests. The ANOVA of the static test method data detected a significant age effect, but no effect by species for LC50's calculated by each of the three techniques (Table 5), and a similar analysis of the flow-through test data showed no significant age nor species effect by any of the computational approaches (Table 5).

Duncan's procedure was used for pairwise comparisons of the age groups from the static tests with chlorpyrifos. For the probit LC50, day-of-hatch group was significantly different from the 7-day-old and 14-day-old groups, but not from the 28-day-old group (Table 6). The 28-day and 14-day groups differed from each other. Both the moving average and binomial LC50 data showed significant differences between the day-of-hatch group and the 7-day-old group and between the day-of-hatch and the 14-day-old groups. No pairwise comparisons were necessary for the flow-through test method since the ANOVA showed no significant effects.

Table 3. Three-way analysis of variance of 96-hr LC50s computed by probit analysis, moving average, and binomial test for three atherinid fish species, of four ages, exposed by two test methods to chlorpyrifos or thiobencarb

Source	d.f.[a]	Probit		Moving average		Binomial	
		F	Pr > F	F	Pr > F	F	Pr > F
Chlorpyrifos							
Species	2	2.62	0.153	1.66	0.267	2.50	0.163
Age	3	10.62	0.008	8.15	0.015	7.54	0.019
Method	1	103.31	0.0001	74.24	0.001	52.61	0.0003
Species × Age	6	1.86	0.235	1.63	0.284	1.15	0.433
Species × Method	2	0.12	0.893	0.17	0.848	0.08	0.924
Age × Method	3	8.29	0.015	6.78	0.024	5.54	0.037
Error	6						
Thiobencarb							
Species	2	6.33	0.033	5.80	0.040	3.18	0.115
Age	3	13.53	0.004	14.26	0.004	8.34	0.015
Method	1	13.75	0.010	14.32	0.009	8.81	0.025
Species × Age	6	3.51	0.076	3.99	0.058	2.87	0.112
Species × Method	2	0.01	0.990	0.01	0.994	<0.01	0.999
Age × Method	3	0.20	0.891	0.06	0.980	0.22	0.880
Error	6						

[a] Degrees of freedom (n − 1)

Table 4. Duncan's procedure (α = 0.05) for simple pairwise comparisons of fish species and ages exposed to thiobencarb. Mean 96-hr LC50 values (μg/L) for species or ages commonly underscored are not significantly different

	Species			Age (days)			
	M. peninsulae	*M. menidia*	*L. tenuis*	Hatch	7	14	28
Probit	386.5	496.0	572.6	373.8	372.3	485.8	708.2
Moving average	390.3	482.9	565.6	371.3	359.8	484.8	702.3
Binomial	603.9	522.8	407.8	389.2	350.3	551.2	755.2

Discussion

The three atherinid fishes tested are sensitive indicators of thiobencarb and chlorpyrifos toxicity. Statistically significant (α = 0.05) differences were detected among species, age groups and test methods, although the range of 96-hr LC50 values was, with few exceptions, limited. The acute toxicity of chlorpyrifos was two orders of magnitude greater than that of thiobencarb. Sensitivity to test materials also varied with age: acute sensitivity was greatest at 7 days for thiobencarb, and at 14 days for chlorpyrifos. These results suggest that predictions of field toxicity with any of the three species would provide a reasonable estimate of possible environmental risks, especially if 7- or 14-day-old larvae are used in either static or flow-through tests.

Sensitivities of 7- and 14-day-old larvae of each species (96-hr LC50's ranging from 199 to 840 μg/L) in our study were substantially greater than a 96-hr LC50 of 1,370 μg thiobencarb/L reported for 48-day-old sheepshead minnows (*Cyprinodon variegatus*) in flowing seawater tests (Schimmel *et al.* 1983). Similar results by Borthwick and Walsh (1981) estimated a 96-hr LC50 of >1,000 μg thiobencarb/L for 28-day-old *C. variegatus* fry in static tests.

Several fingerling freshwater fishes were also less sensitive to thiobencarb than the species we tested. Johnson and Finley (1980) reported static 96-hr LC50 values of 1,200 μg/L for rainbow trout (*Salmo gairdneri*), 2,300 μg/L for channel catfish (*Ictalurus punctatus*) and 2,500 μg/L for bluegills (*Lepomis macrochirus*). In the present study, 96-hr LC50's ranged from near unity to almost two times greater than the computed values for our least sensitive fish (*Menidia peninsulae*, 28-day old larvae: 96-hr LC50 = 1,405 μg/L) exposed to thiobencarb in static tests (Table 2). Flowing water tests yielded estimates of thiobencarb sensitivity similar to the static tests. Our least sensitive fish (*Menidia peninsulae*, 28-day-old larvae: 96-hr LC50 = 865 μg/L) was 1.4 to

Table 5. Two-way analysis of variance for static and flow-through tests with chlorpyrifos

Source	d.f.[a]	Probit F	Probit Pr > F	Moving average F	Moving average Pr > F	Binomial F	Binomial Pr > F
Static tests							
Species	2	0.57	0.591	0.26	0.780	1.08	0.396
Age	3	10.93	0.008	8.40	0.014	7.36	0.020
Error	6						
Flow-through tests							
Species	2	1.39	0.320	1.35	0.329	1.51	0.295
Age	3	1.72	0.263	1.63	0.279	2.48	0.159
Error	6						

[a] Degrees of freedom (n − 1)

2.9 times more sensitive than the freshwater static tests of Johnson and Finley (1980) using rainbow trout, channel catfish, and bluegill fingerlings (96-hr LC50: 1,200 to 2,500 μg/L).

A comparison of our findings with published data for other marine species indicates that 7- and 14-day atherinids are excellent indicators of chlorpyrifos toxicity. In static tests, Borthwick and Walsh (1981) reported a 96-hr LC50 of 270 μg/L chlorpyrifos for 28-day old (*C. variegatus*). This LC50 value is 40 times greater than the least sensitive fish (*L. tenuis* newly hatched larvae: 96-hr LC50 = 6.7 μg/L) (Table 2). Comparison of results for flow-through tests indicate that the 96-hr LC50 of 136 μg/L for 35-day-old *C. variegatus* (Schimmel *et al.* 1983) is 44 times greater than the *least* sensitive fish (*M. menidia,* 28-day-old larvae: 96-hr LC50 = 3.1 μg/L). Although the divergence in responses of other marine fishes is not as striking as with *C. variegatus* in all comparisons each of the atherinid fishes are as sensitive (or more sensitive) indicators of chlorpyrifos toxicity. Schimmel *et al.* (1983) reported 96-hr LC50s of 5.4 μg/L for striped mullet (*Mugil cephalus*), 4.1 μg/L for longnose killifish (*Fundulus similis*) and 1.7 μg/L for 53-day old *M. menidia* juveniles (a value comparable to data reported in Table 2). Striped bass, *Morone saxatilis,* with a 96-hr LC50 of 0.58 μg chlorpyrifos/L (Korn and Earnest 1974) is the only species that has a sensitivity approaching that of early atherinid life stages.

Acute static 96-hr LC50s for several fingerling freshwater fishes (Johnson and Finley 1980) indicate a broad range of sensitivity to chlorpyrifos: 18 μg/L for cutthroat trout (*Salmo clarki*), 7.1 μg/L for rainbow trout (*Salmo gairdneri*), 98 μg/L for lake trout (*Salvelinus namaycush*), 280 μg/L for channel catfish (*Ictalurus punctatus*), and 2.4 μg/L for bluegill (*Lepomis macrochirus*). Chlorpyrifos toxicity to bluegill was 15-fold greater at 29°C than at 13°C, and the 96-hr LC50 for rainbow trout at 18°C was twice that at 2°C. Macek *et al* (1969) exposed rainbow trout at 1.6 and 12.7°C and found the 96-hr LC50s to be 7.1 and 51 μg/L, respectively.

Table 6. Duncan's procedure (α = 0.05) for simple pairwise comparisons of age groups of fishes exposed in static tests to chlorpyrifos. Mean 96-hr LC50 values (μg/L) for age groups commonly underscored are not significantly different

	Age (days) 14	7	28	Hatch
Probit	2.00	2.50	3.53	4.73
Moving average	1.97	2.50	3.53	4.86
Binomial	2.03	2.47	3.63	5.40

According to Stephan (1977), probit analysis is the least conservative method and is generally preferred when test results provide at least two partial kills one on either side of 50% mortality. When at least two partial kill responses is achieved, moving average and probit produce similar LC50's. However moving average is simpler to compute and is equally reliable with data sets containing one partial bracketed by 0% and 100% kills. Further, it will compute an LC50 without confidence limits when no partial kills occur. The binomial test can calculate a statistically sound test result with no partial kills where probit or moving average are inappropriate. Although the binomial test usually estimates an LC50 comparable to probit and moving average, its wider more conservative confidence interval is not based on 95% confidence limits. Nevertheless, these limits are considered statistically useful since the level of confidence is above 95% if six or more organisms are exposed to each treatment. The binomial test is an exact method which makes no underlying assumptions other than that the data are dichotomous. It supports argument against a prerequisite for partial kills required by some aquatic

toxicologists (Stephan 1977; Oglesby and Bundrick 1981).

By regressing all LC50s for probit vs moving average, probit vs binomial, and binomial vs moving average methods for each pesticide (Table 2), we found that probit and moving average methods calculated comparable LC50s. But when either probit or moving average LC50s were regressed with the binomial LC50s, some deviation from unity was evident in the plots. These observations were consistent for both chlorpyrifos and thiobencarb.

These simple comparisons among the three computational methods using empirical data agree with conclusions in the literature based on hypothetical data sets. These conclusions state that: (1) probit and moving average methods calculate LC50 estimates that are essentially the same, and (2) binomial LC50 estimates closely agree with probit and moving average LC50 estimates, but the binomial confidence interval is generally much wider than the confidence intervals for the probit and moving average estimates.

Further, significant differences among age groups and species (Table 4) detected by Duncan's procedure for both probit and moving average methods were not detected for the binomial method. This inconsistency is probably due to the binomial method being the least precise point estimator among the three computational methods.

We conclude: (1) the three species of Atherinidae were similarly sensitive and any one may be selected as representative of the family based on our toxicity test results; (2) generally, sensitivity was highest for fish reared for 7 days posthatch for thiobencarb, and for 14 days for chlorpyrifos; (3) as expected, differences in LC50s between static and flow-through 96-hr exposures indicate that flow-through tests are more sensitive for these compounds (this difference being greater for chlorpyrifos than for thiobencarb); (4) probit and moving average methods estimate LC50s that are essentially the same for empirical acute toxicity data.

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